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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Dong et. al. § ART UNIT: 1636
§
FILED: August 27, 2001 § EXAMINER: Akhavan, Ramin.
§
SERIAL NO.: 09/600,521 §
§ CONFIRMATION NO. 7109
FOR: Method and Composition For Treating Tumors By Selective Induction of Apoptosis § DOCKET: D6579

MS Non-Fee Amendment
Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313

DECLARATION UNDER 37 C.F.R.§ 1.132

Dear Sir:

I, James S. Norris, hereby state as follows:

I am an inventor of the above-referenced U.S. patent application Serial No. 09/600,521 and I am aware of the contents of the Office Action, mailed May 17, 2005. In this Office Action, an issue relating to the patentability of the claimed method for inducing death in cancer cells is the degree of enablement provided by the Applicant's specification with regard to the efficacy of the claimed method *in vivo*. The following data is presented as evidence of enablement commensurate with the scope of the claims.

In vivo studies performed in my laboratory to determine the effect of AdFasL-GFP_{TET} on tumor growth

A. UM-SCC14A cells were seeded in 150mm plates overnight to reach 70-80% confluence and harvested using cell stripper after washing with

PBS. Cells were washed twice with PBS and centrifuged at 500g x 5 min and resuspended in serum free media (SFM) at a concentration of $1x10^7$/100ul. 100ul of the suspended cells were injected subcutaneously into the right flank of the nude female nu/nu mouse. Animals were monitored twice a week for tumor growth and the size of the tumors were measured in three perpendicular dimensions using calipers and described as tumor volume. It was calculated using the formula [π/6 x height(H) x width(w) x length (L)] . When the tumor size reached 70-100 mm^3, treatment with AdFasL-GFP_{TET} or control (PBS) was initiated. AdFasL-GFP_{TET} (10^9 IU) was injected in different sites of the tumor in 50uL volumes. Injection was carried out every 72 hours for 3 injections. Animals were sacrificed when the tumor reached 2000 mm^3. The number of animals in each of the groups was 6.

As shown in Figure 1A, treatment with AdGFP-FasL caused a complete regression of tumor compared to control untreated cells. Figure 1B compares the overall tumor growth rate in untreated and treated mice. Treatment with AdFasL-GFP led to significant regression of the growing tumors in 100% of the mice with complete remission in 50% of the tumors. None of the animals died in this experiment.

B. 1 x 10^6 prostate cancer (DU145) cells were injected subpannicularly into the right flank of 7-9 week old, male nu/nu mice (obtained from NIH). The treatment was initiated when the tumors reached 70-100 mm^3. Tumors were measured using calipers and volume was calculated using the formula ($0.5236 \times r_1^2 \times r_2$ ($r_1<r_2$)). Untreated mice received no treatment at all

whereas control mice were treated with a solution containing 10% ethanol, 30% cremophor and 60% saline. AdFasL GFP_{TET} in PBS at 2 X 10^9 pfu was injected in 50ul volume in 10ul increments in different parts of the tumor. Virus was injected twice at 10 and 82 hours and there were 7 mice in each of the groups.

Figure 2 compares the overall tumor growth rate in control and (AdFasL-GFP_{TET}) treated mice. It was observed that treatment with AdFasL-GFP led to significant regression of the growing tumors. None of the animals died in this experiment. Based on the results of these studies, a person having ordinary skill in this art would have a reasonable expectation that the claimed methods of the instant invention will be effective *in vivo*.

I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 or Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 8/16/05

Dr. James S. Norris

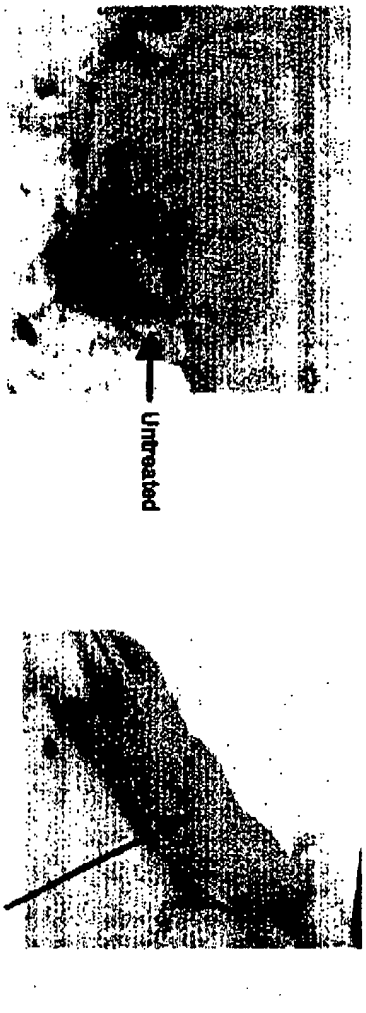

Fig. 1A

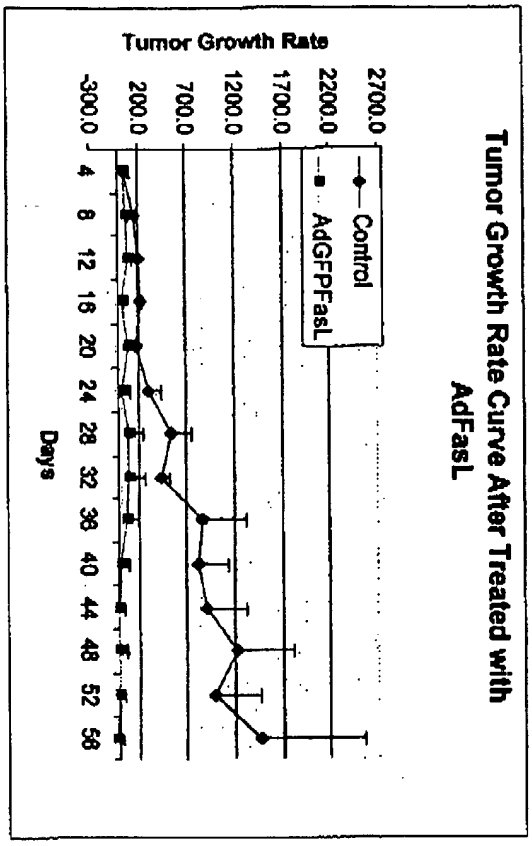

Fig. 1B

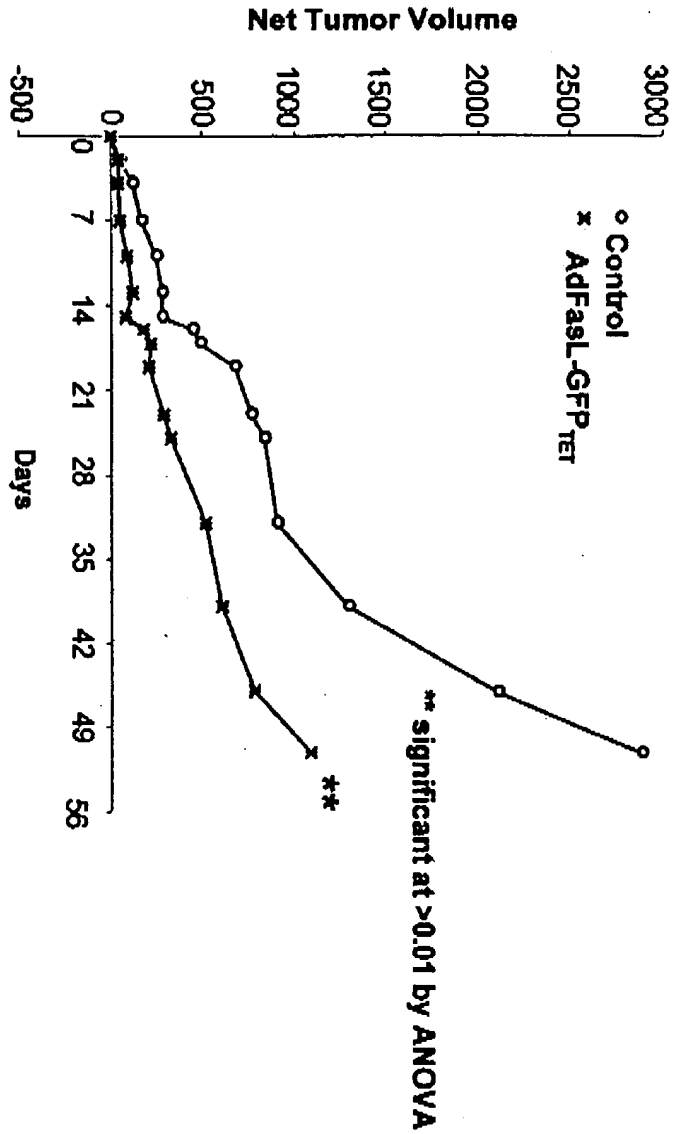
Net Tumor Volume
-500
0
500
1000
1500
2000
2500
3000
0
7
14
21
28
35
42
49
56
Days
° Control
× AdFasL-GFP TET
**
** significant at >0.01 by ANOVA

Fig. 2